Seroepidemiological Survey of Chlamydia Trachomatis in Patients Attending Pre and Post Natal Clinics in Lagos Nigeria

L.E. Okoror[1,*], S.A. Omilabu[2], P.O. Orue[1] and G. Ajayi[3]

[1]Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, PMB 14. Ekpoma Nigeria; [2]Virology Unit, Department of Medical Microbiology and parasitology, College of Medicine of University of Lagos. Idi-araba. Lagos Nigeria and [3]Department of Gynaecology and Obstetric, Lagos University Teaching Hospital, Idi-araba. Lagos Nigeria

Abstract: Of the 245 patients screened for the presence of complement fixing antibody to *Chlamydia trachomatis,* 89 (35.74%) tested positive while 104 (42.5%) of the patients were weakly positive and 4 (1.61%) were non-specific. Age group distribution showed that the highest number of patients testing positive to *Chlamydia* complement fixing antibody was in the age range of 31-36 year for both males and females while the lowest number of the age range testing positive was between 19-20 and 43-48 years of age for both sexes. One out of 4 umblical cord fluids tested positive along with the mother, father and baby a month after delivery. All males who accompanied their spouses to the clinics were also tested for *Chlamydia* complement fixing antibody and all the males involved in active cigarette smoking tested positive. The highest antibody titre was in the age range of 31-36 years. There was a four fold rise in antibody titre for nearly all the patients except for a few who had their antibody level dropping, most probably because they were in the convalescence stage. This study concludes that the infection is endemic especially in such a very delicate population as anti-natal patients. This is because of the risk of mother to child transmission.

Key Words: *Chlamydia trachomatis,* complement fixation test, complement fixing antiboby, *Chlamydia,* prenatal, postnatal.

INTRODUCTION

Trachoma is the prototype Chlamydial infection and was recognised in the ancient times of Greece and Egypt. They were thought to be viruses and were referred to as large viruses. Three species were first recognised which included *Chlamydia trachomatis, Chlamydia pneumoniae and Chlamydia psittaci* [1]. They belonged to the order *Chlamydiales* and family *Chlamydiaceae.* The wide spread importance and frequencies of genital and infant chlamydial infection were first appreciated in the 1960's [2]. *Chlamydia trachomatis* is composed of two biovars, the lymphogranuloma venereum agents and the ocular serotype which may be distinguished by antigens well described seroprevalent with distinctive outer membrane proteins [3]. Members of this family have a group distinctive antigen. *Chlamydia psittaci* infections are common among those handling poultry and psittacine birds while *Chlamydia pneumoniae* which is unique to humans causes upper and lower respiratory tract infections. It also causes conjunctivitis, along with pneumonia which may not be clinically distinguishable from those by *Mycoplasma pneumoniae* [4, 5].

Chlamydia lymphogranuloma venereum is a biotype of trachomatis causing a generalised infection transmitted venerally which is of worldwide distribution and has been described as 6th V.D. *Chlamydia trachomatis* causing mucopurulent cervicitis, urethritis, endometritis, salpingitis, perihepatitis (Fitz-High-curtis syndrome), and latter post partum endometritis. At least one third of infected females have no symptoms [6]. Young children are particularly vulnerable to infection. Transmission is usually by contact with formitis in an unhygienic environment [7]. *Chlamydia trachomatis* could also cause pharyngitis in children and adults. Diseases due to *Chlamydia trachomatis* are more common in the tropics. Evidences are accumulating that most infections occur during birth from infected genital passages [8]. *Chlamydia trachomatis* similar serotype has been isolated from the eyes of a baby, vagina of the mother and urethra of the father [7]. *Chlamydia trachomatis* is common in pregnant women in developed and developing countries and the prevalence varies with age [6]. Approximately 75% of infants born to vaginal infected women become infected. And the infection may remain latent several months after birth [2]. Less commonly, infants born by caesarean sections may also be infected. The anatomic sites most commonly infected in infants is the conjunctiva, which often manifests as purulent conjunctivitis and the nasopharynx, often presenting as chronic congestion. The most serious manifestation of perinatal chlamydial infection is pneumonia, which may range in severity from mild to fatal if untreated [6]. In many cases *Chlamydia trachomatis* is the most common cause of purulent conjunctivitis in the first few months of life and of a febrile pneumonia in the first 3 months of life [6, 9].

In Nigeria reports have been incriminated in ectopic pregnancy [8]. Studies still remain scanty when relating to the scourge of this "silent" organism worldwide. The pathogen has been given little or no attention in Nigeria and has

*Address correspondence to this author at the Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, PMB 14. Ekpoma Nigeria; E-mail: LEOkoror@gmail.com

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cause damages unnoticed and/or unchallenged. This study sought to establish the prevalence of this organism in pregnant women and partly the maternal-child relationship and its possible involvement in infertility.

MATERIALS AND METHODS

Sample Collection

Blood samples were collected from pre and postnatal patients attending diagnosis at the College of Medicine of the University of Lagos, Idi-araba, Lagos Nigeria. This included pregnant and non pregnant women along with their spouses. Women complaining of frequent miscarriages and infertility were also included in this study. Samples were collected between 1996 and 1997. Blood samples were collected by venal puncture and stored in sterile dry venoject vacutaneers and allowed to clot. The sera were separated by spinning the blood in a centrifuge (Hitutich Universal) at 3,000rpm. The sera were then separated and stored away in a -20^{O}C refrigerator till used. Paired serum samples were also collected. The paired serum samples collected from all the patients testing positive were collected after two weeks of the first test to confirm rising antibody titre since it has been reported that a fourfold rise in antibody titre is diagnostic for complement fixation test. Paired serum samples are a second sample collected from the same subject two weeks after the first sampling to confirm seroconversion to *Chlamydia trachomatis* antibody. And hence validates the specificity and sensitivity of the results.

Complement Fixation Test

All the sera obtained were subjected to complement fixation test (CFT). The complement fixation test belongs to complex serological reactions. It requires an antigen, an antibody and a complement (the first system), sheep red blood cell and haemolytic serum (the second system). The principle is that when there is antigen antibody reaction, and complement is added to the system, it binds to the antigen-antibody complex. If there is no antigen antibody reaction, the complement will not bind because there is no antigen-antibody complex and hence there will be free complement. The free complement will then lyse the sensitized sheep red blood cells when the second system is added and then the test is said to be negative. Okoror *et al*. [10] used complement fixation test to study the prevalence of *Chlamydia* in South Eastern Nigeria. Materials used for complement fixation test included sheep red blood cell, veronal buffer diluents which were commercially obtained as CFT tablet (Oxoid), hemolysin which was titrated to reach the required dilution [11] and complement which was commercially obtained as preserved guinea pig serum (Welcome Research Laboratory, England). The antigen used was that of *Chlamydia trachomatis* and was originally isolated from a patient using embryonated eggs. The antigen was titrated using the method of Krivoshein [11]. Complement fixation test as adapted by microtiter plate was performed as described by Krivoshein [11].

Antibody titration was also carried out for those that returned for paired serum sample collection. Antibody titration was carried by first doing a doubling dilution of the sera using veronal buffer as diluent in a U-shaped microtitre plate starting with a drop of the sera with the aid of microdiluters. And starting from 1:8 dilution to 1:1024. This was followed by the addition of a drop of antigen to all the wells followed by complements. The mixtures were then incubated for 90 mins at 37^{o}C. A drop of sensitized sheep erythrocyte was added and re-incubated at 37^{o}C for 30 mins and vortexed at every 10 mins interval. The U-shaped microtitre plates were then kept in a 4^{o}C refrigerator for 20 mins to allow free cells settle and the microtitre plates were brought out and read on an optical reader.

Data Analysis

Results were statistically analysed using the statistical software package SPSS version 11. Chi square, student t test as well as regression analysis was used in the analysis.

RESULT

A total of 249 samples were screened for *Chlamydia* complement fixing antibody of which 120 (47.9%) were males patients and 125 females patients while the rest were umbilical cord fluids. Of the males screened, 59 (23.8%) were positive while 61 (24.11%) were negative. 78 (31.4%) were positive for females while 47 (18.7%) were negative. In all, 137 (55.2%) were positive (this include those that were weakly positive) and 108 (42.5%) negative. The age group distribution showed that 7 males within the ages of 19-24 were positive while 5 within the ages of 25-30 years were positive, 25 in the ages of 31-36 years were positive. Twelve males between the ages of 37-42 were positive while 10 males in the age group 43-48 years tested positive. For females, 9 between 19-24 years of age were positive while 10 tested positive for ages 25-30 years. Forty-three were positive for age group 31-36 and 9 were positive for age 37-42 years. Seven tested positive in age group 43-48 years (Table **1**).

Of the 4 umblical cord tested, one tested positive to complement fixing antibody to *Chlamydia*. One was non-specific (Table **2**).

Of all the 249 patients screened only 33 of them came back for second sample collection and in some there was a four-fold rise in antibody titer while in others there was a drop.

Chlamydia antibody in pre-natal, antenatal and postnatal patients was also tested. Of the 40 patients for pre-natal diagnosis, 70% of them tested positive for antibody to *Chlamydia* while for antenatal, 40% of the total 56 patients tested positive to *Chlamydia* antibody. And for post-natal patients, 20% of the 29 patients tested positive.

The antibody titration of all the positive sera was also carried out and result is shown in Table **3**.

Although there were no clear-cut symptoms, some of the females had problems of vaginal discharge and itches. Of the 78 positive women to Chlamydia Complement Fixing Antibody, 25 were women who have never conceived and 10 had history of miscarriages and other complications during pregnancy. The rest were women who normally conceive but have a history of likelihood of miscarriages and other minor complications. Their spouses also tested positive to Chlamydia Complement fixing antibody except for 19 men whose

Table 1. Distribution of Chlamydia Complement Fixing Antibody in Individuals Attending Pre and Post Natal Diagnosis in Lagos Nigeria

Age Group In Years	Number Positive (%)		Number Negative (%)		Total
	Male	Female	Male	Female	
19-24	7(28)	9(36)	4(16)	5(20)	25
25-30	5(17.9)	10(35.7)	6(21.4)	7(25)	28
31-36	25(19.5)	43(33.6)	33(25.8)	27(21.1)	128
37-42	12(32.4)	9(24.3)	8(21.6)	8(21.6)	37
43-48	10(37)	7(25.9)	10(37)	-	27
Total	**59(24.1)**	**78(31.8)**	**61(24.9)**	**47(19.2)**	**245**

(t=2.285, CI =99%, α=0.084)

Table 2. Prevalence of Chlamydia Complement Fixing Antibody in Umbilcal Cord Fluids

Samples	Sex of Newborn	Test	Antibody Titration
Fluid 1	Male	positive	1:16
Fluid 2	Male	Negative	-
Fluid 3	Female	Negative	-
Fluid 4	Male	positive	Non specific

Table 3. Antibody Titre of Sera Tested Positive To Chlamydia Complement Fixing Antibody

Titer Levels:	>1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
No. of Patients	58	27	38	28	17	8	9	1

wives had normal pregnancy but with minor complications. The average positive result was 22.9 while the standard error was 10.271. The t value using the paired t test was 2.285.

One of the four umbilical cord fluids of a baby tested positive along with the mother and the father.

The paired serum samples were also tested after a period of one month after the first sample collection, only 33 patients turned up for the second time. There was a four fold rise in titre for most of the sera while others showed a depleting antibody. Table **4** shows the seroconversion pattern to *Chlamydia* antibody.

DISCUSSION

The high prevalence of positive result in this study agrees with the fact that *Chlamydia trachomatis* is more prevalent in the tropics since *Chlamydia trachomatis* antigen was used

Table 4. Seroconversion Pattern to Chlamydia Complement Fixing Antibody

Samples	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
1st	1:8	1:8	1:8	1:8	1:16	1:8	1:64	1:16	-	-	1:8	1:8	1:32	1:64	-
2nd	1:64	1:64	1:32	1:128	1:32	1:64	1:8	1:16	-	-	1:8	1:8	1:256	1:512	-

Samples	A16	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26	A27	A28	A29	A30
1st	1:128	1:32	1:16	1:128	1:32	1:32	1:16	1:16	1:8	1:8	1:16	1:8	1:8	1:16	-
2nd	1:8	1:8	1:16	1:512	1:128	1:16	1:64	1:64	1:64	1:64	1:256	1:16	1:512	1:256	-

and this is in agreement with Patiel *et al.* [12]. The inclusion of males and patients with infertility cases and miscarriages is justified by the fact that *Chlamydia trachomatis* has previously been incriminated in low sperm count and female infertility and miscarriages [6]. This study also reveals that the prevalence of *Chlamydia* was influenced by age with older men showing higher number of *Chlamydia* cases. This high number of positive cases may not just be attributed to *Chlamydia trachomatis* alone since complement fixation test was used in this study and detected the group reactive antigen of *Chlamydia.* The high positive cases in older men may have been influenced by other *Chlamydia* spp. especially *Chlamydia pneumoniae* which is not particularly under study here. Such factors like smoking may have influenced this trend in older men as earlier reported by Patiel *et al.* [12] and Hann and Gohibjatnikov [13]. The antibody titer for males in the older ages was less in men than as compared to women, there are lots of other reasons which can bring down immunity in older men and this include a reduction in activities as well as aging. More men between the ages of 31-36 years have higher number of positive cases and this may not be unconnected with the fact that men in this age group are sexually active as against their female counterparts or spouses who customarily are not allowed to engage in extra matrimonial sexual activities. It is also in this age group that females have higher number of positive results and this is likely due to the fact that the population sampled is the child bearing population and this age group is mostly associated with child-bearing. Since only pre and postnatal patients and their spouses were screened. The fact that nearly all the patients screened showed no clear-cut symptoms despite high antibody titer may not be unconnected with latent infection of *Chlamydia* spp. as earlier reported by Ogawa *et al.* [14]. It has also been reported that the fact that an individual comes up negative with complement fixation test does not rule out infection as complement fixing antibodies will always develop late in the infection and even they remain in the acute phase may not easily be detected hence negative result in this study may not actually all be negative. Schachter and Grossman [2] reported that the prevalence of *Chlamydia* was inversely proportional with age, which suggests why there was a sharp drop in antibody titer as age increased in this study.

Sex distribution shows that more females have higher antibody to *Chlamydia* than their male counterparts. This might be due to the involvement of pregnancy in inducing hormonal secretions that may make the females more vulnerable to infection as there was a four fold rise in antibody tier in most of the females sampled. Statistical analysis justified both the age groups and sampling and also validated the results obtained since t calculated was higher than tabulated t. This showed that *Chlamydia trachomatis* may be a source of complications in pregnancy. It could also be a source of complications been experienced by those females and their spouses attending pre-natal diagnosis.

The positive samples from the umbilical cord of a new born as well as the mother testing positive to *Chlamydia* complement antibody, clearly support neonatal infection, though *Chlamydia* does not cross the placenta but is transmitted to new borns during birth *via* infected birth canals. Another confirmation of neonatal transmission of the infection is that three months after the mother still tested positive to *Chlamydia* complement fixing antibody as well as the father and the infant. A female with vaginal discharge which has defiled most commonly used antibiotic also tested positive to *Chlamydia* complement fixing antibody. This supports earlier report by Strickland [15] that *Chlamydia* is responsible for over 40% non-gonococcal urethritis. The four fold rise in titer of antibody suggests recent infection. Where the drop in antibody titer may be connected with the fact that samples may have been collected while the patients were convalescing. This also goes for those, who later turned negative.

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